

Fig. 1

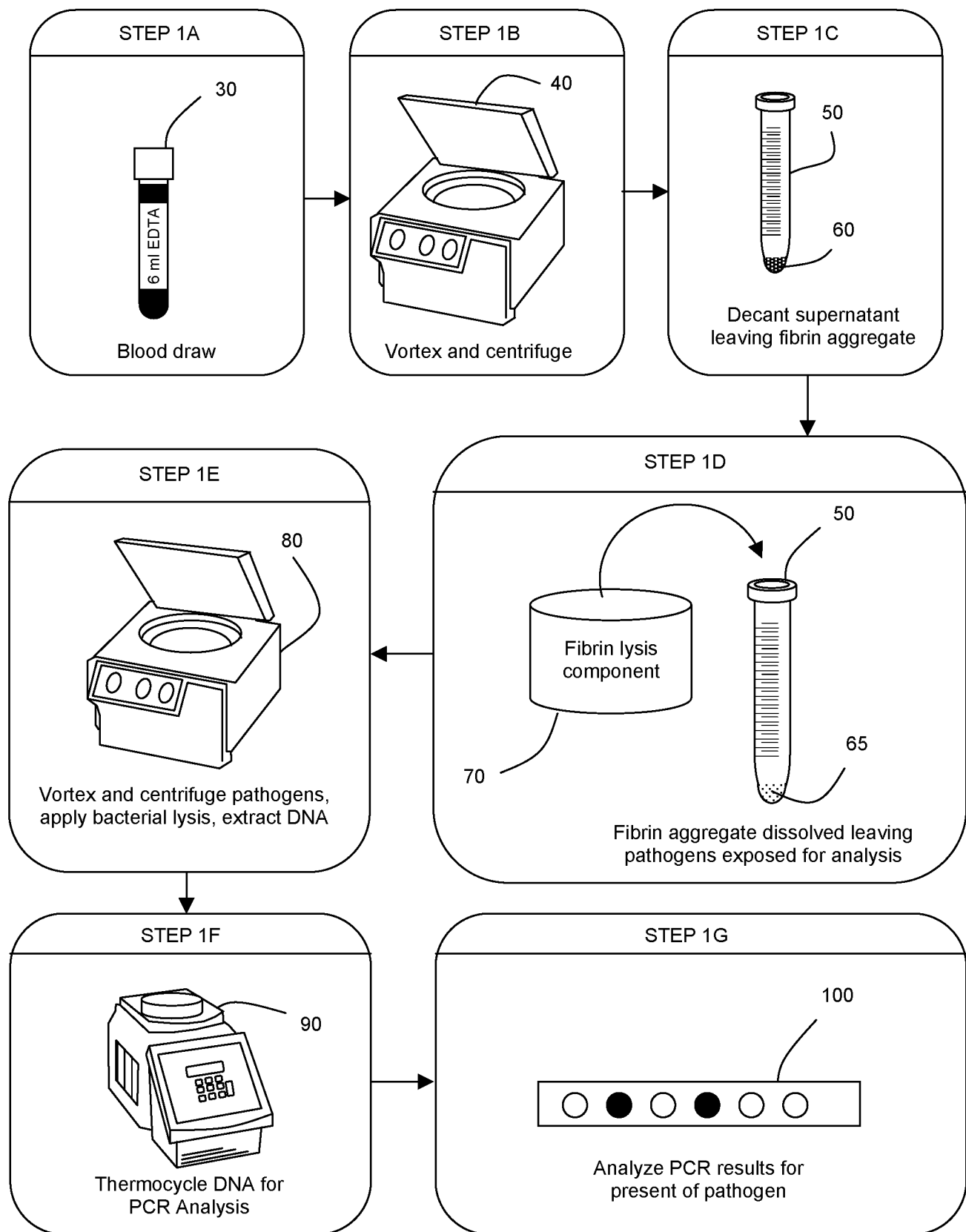


Fig. 2

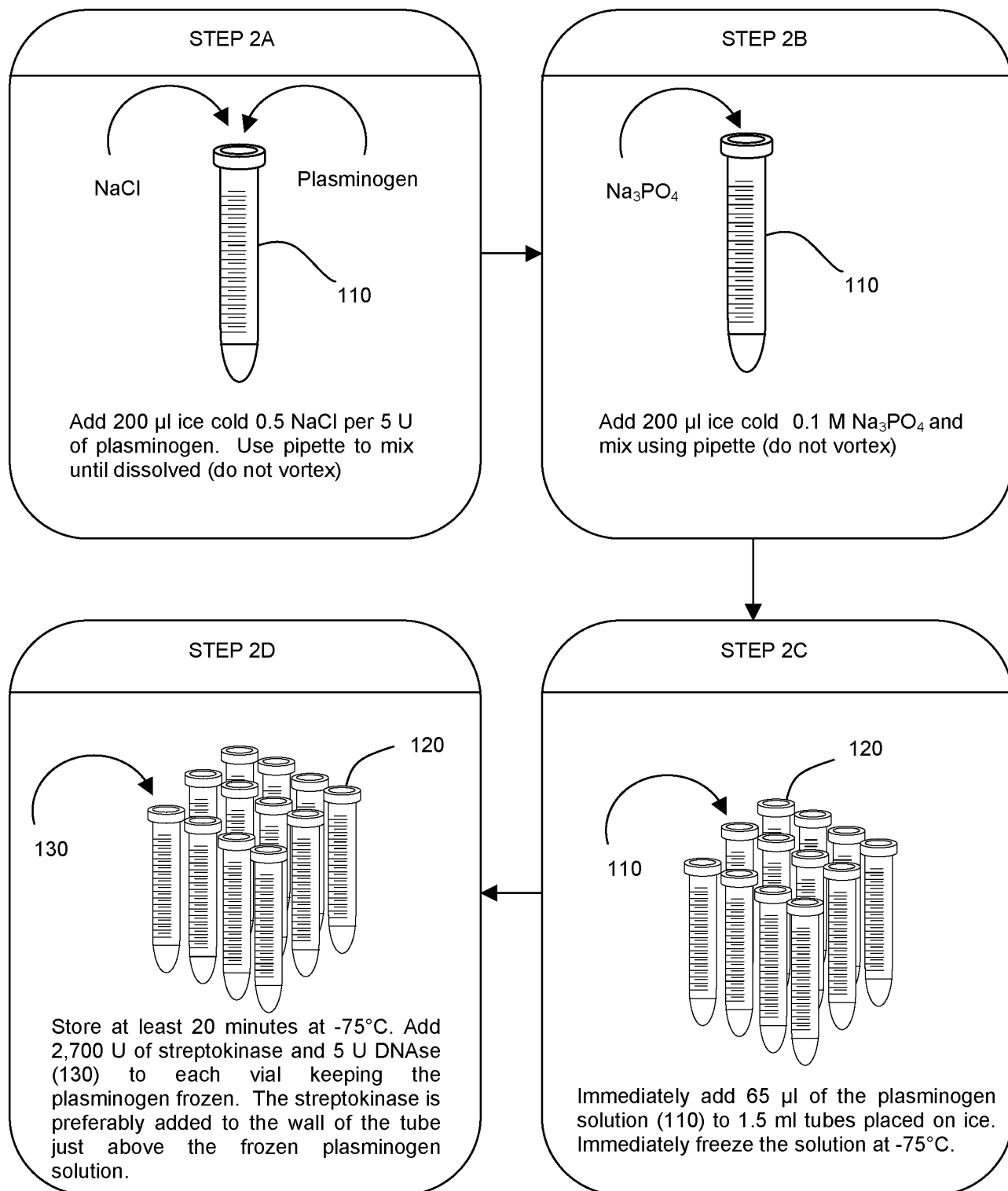


Fig. 3

***Bacillus anthracis* Blood Protocol Data Set**

Sample Number	pXO2 Primer / Probes - Crossing Point on Light Cycler	Genomic Primer / Probes - Crossing Point on Light Cycler	Ave. Calculated CFU/ 6 ml of blood	Comments on Sample Type All Samples Tested 2 Days Post Spiking
M3200253BA1	36.75	37.76	13.75	Spiked Positive
M3200253BA2	36.59	37.86	13.75	Spiked Positive
M3200253BA3	35.97	38.10	13.75	Spiked Positive
M3200253BA4	37.26	39.53	13.75	Spiked Positive
M3200253BA5	35.36	40.11	13.75	Spiked Positive
M3200253BA6	36.35	45.19	13.75	Spiked Positive
M3200253BA7	36.62	38.64	13.75	Spiked Positive
M3200253BA8	37.04	39.51	13.75	Spiked Positive
M320020BA9	0.00	0.00	0.00	Blank
M/3200226BA1	37.16	39.35	1.38	Spiked Positive
M/3200226BA2	36.79	40.28	1.38	Spiked Positive
M/3200226BA3	37.92	39.94	1.38	Spiked Positive
M/3200226BA4	37.49	40.16	1.38	Spiked Positive
M/3200226BA5	39.66	40.26	1.38	Spiked Positive
M/3200226BA6	39.31	41.19	1.38	Spiked Positive
M/3200226BA7	38.48	40.73	1.38	Spiked Positive
M/320020BA8	0.00	0.00	0.00	Blank

Fig. 4

***Bacillus anthracis* Blood Protocol Data Set: Comparison of Blood from Two Different Individuals and Evaluation of Blood Sample Age**

Sample Number	pXO2 Primer / Probes - Crossing Point on Light Cycler	Genomic Primer / Probes - Crossing Point on Light Cycler	Ave. Calculated CFU/ 6 ml of blood	Comments on Sample Type All Samples Extracted 84 Days Post Spiking
V210253BA1	37.73	39.81	10.5	Blood Donor #1
V210253BA2	36.74	39.05	10.5	Blood Donor #1
V210253BA3	36.51	37.99	10.5	Blood Donor #1
V210253BA4	38.12	39.79	10.5	Blood Donor #1
V21020BA5	0.00	0.00	0.00	Blank
M210253BA1	37.86	39.81	2.25	Blood Donor #2
M210253BA2	37.84	39.22	2.25	Blood Donor #2
M210253BA3	37.24	38.52	2.25	Blood Donor #2
M210253BA4	38.68	39.33	2.25	Blood Donor #2
M21020BA5	0.00	0.00	0.00	Blank

Fig. 5

***Bacillus anthracis* Blood Protocol Data Set: Evaluation of Blood Protocol by a Department of Health Laboratorian**

Sample Number	pXO2 Primer / Probes - Crossing Point on Light Cyclor	Genomic Primer / Probes - Crossing Point on Light Cyclor	Ave. Calculated CFU/ 6 ml of blood	Comments on Sample Type: All Blood Samples Same Batch as in Table 1
M3200256BA1L	38.81	39.93	13.75	Spiked Positive
M3200256BA2L	36.10	39.26	13.75	Spiked Positive
M/3200223BA3L	36.77	38.58	1.38	Spiked Positive
M320020BA4L	0.00	0.00	0.00	Blank

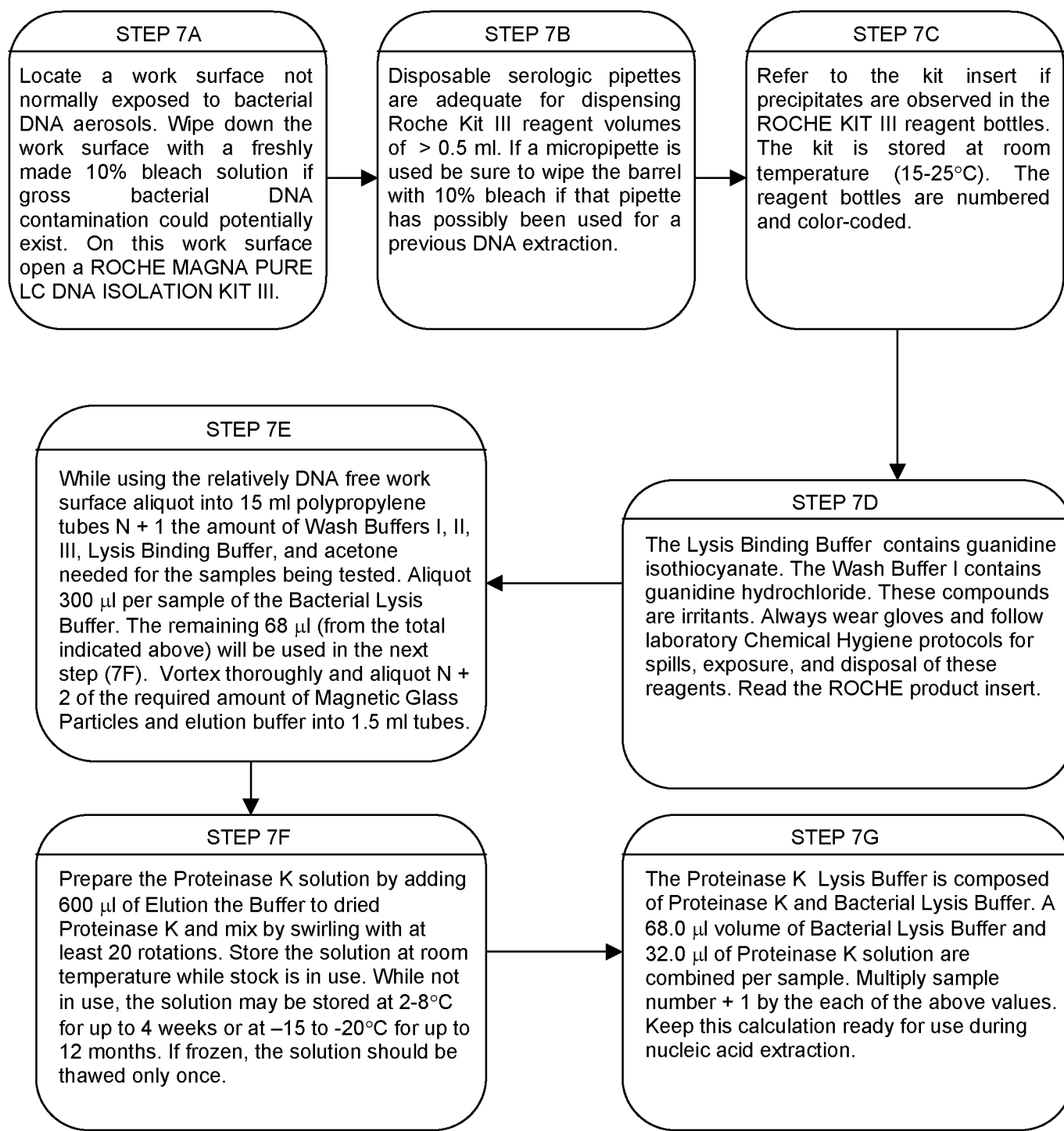
Fig. 6

***Yersinia pestis* Blood Protocol Data Set**

Sample Number	YP 2 Primer / Probes - Crossing Point on Light Cyclor	YP 9 Primer / Probes - Crossing Point on Light Cyclor	YP12 Primer / Probes - Crossing Point on Light Cyclor	YP 16 Primer / Probes - Crossing Point on Light Cyclor	Ave. Calculated CFU/ 6 ml of blood	Comments on Sample Type All Samples Extracted 2 Days Post Spiking
M3180251EYP1	0.00	0.00	0.00	37.97	12.0	Spiked Positive
M3180251EYP2	0.00	47.01	0.00	0.00	12.0	Spiked Positive
M3180251EYP3	41.56	0.00	0.00	40.29	12.0	Spiked Positive
M3180225EYP4	0.00	0.00	0.00	38.98	24.0	Spiked Positive
M3180225EYP6	40.20	44.01	39.66	37.60	24.0	Spiked Positive
M3180251FYP7	0.00	46.15	0.00	39.79	48.0	Spiked Positive
M3180251FYP8	40.48	43.59	41.70	35.47	48.0	Spiked Positive
M3180251FYP9	40.20	41.88	38.67	34.23	48.0	Spiked Positive
M318020YP10	0.00	0.00	0.00	0.00	0.00	Blank

Fig. 7

## Setup of Extraction Reagents



## Bacterial Recovery and Fibrin Lysis

Fig. 8

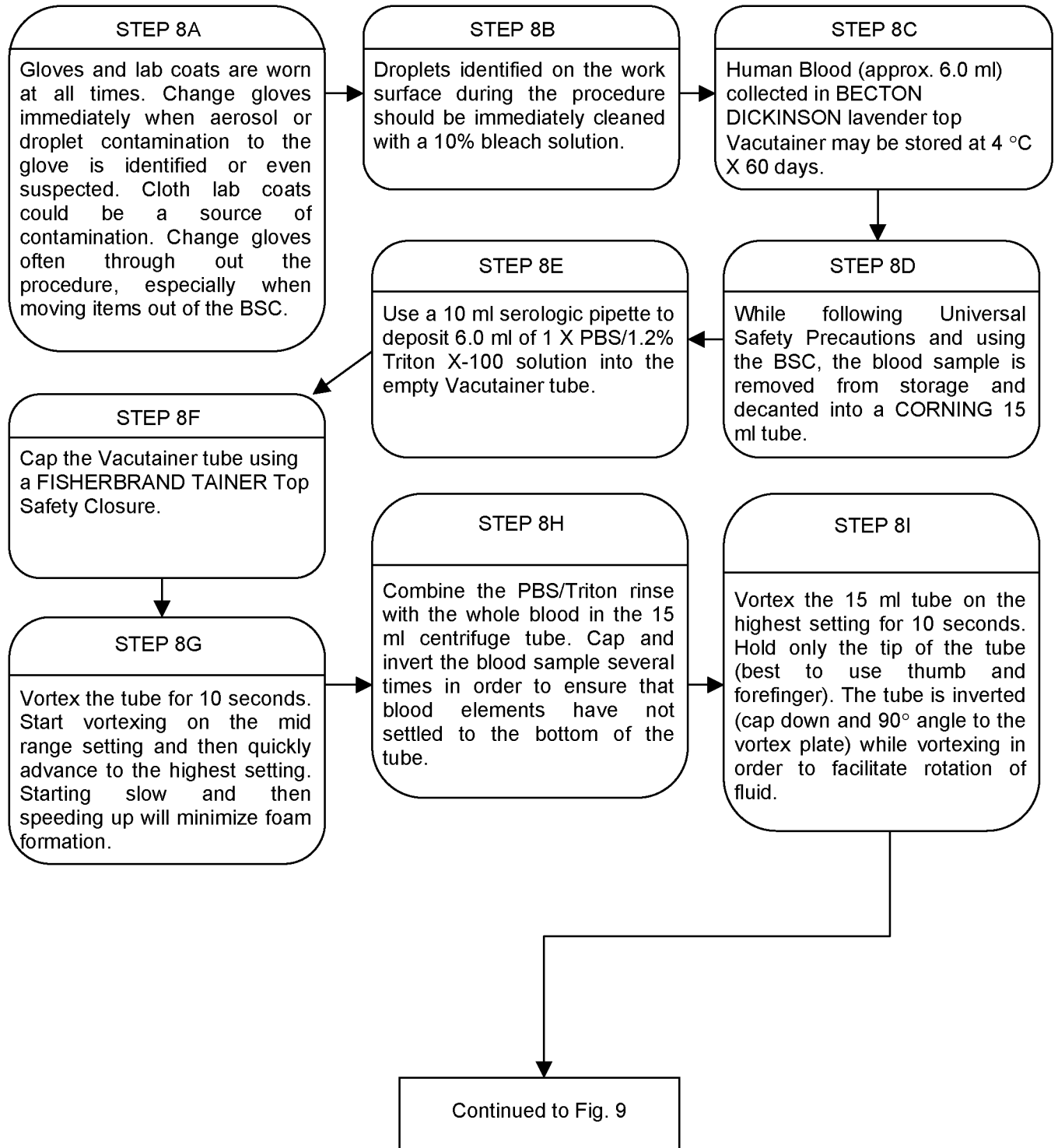


Fig. 9

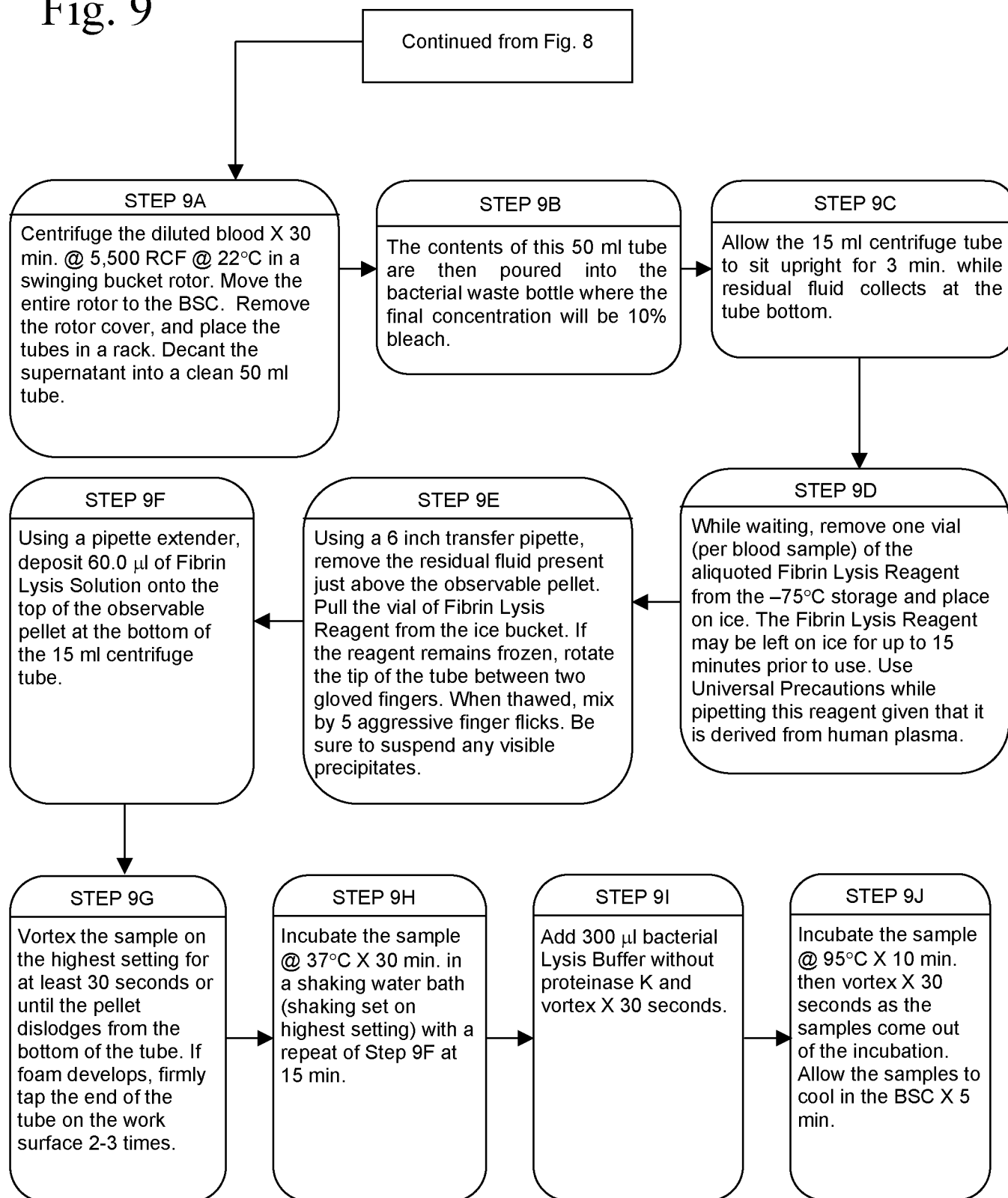




Fig. 10

## Bacterial Lysis and Nucleic Acid Extraction

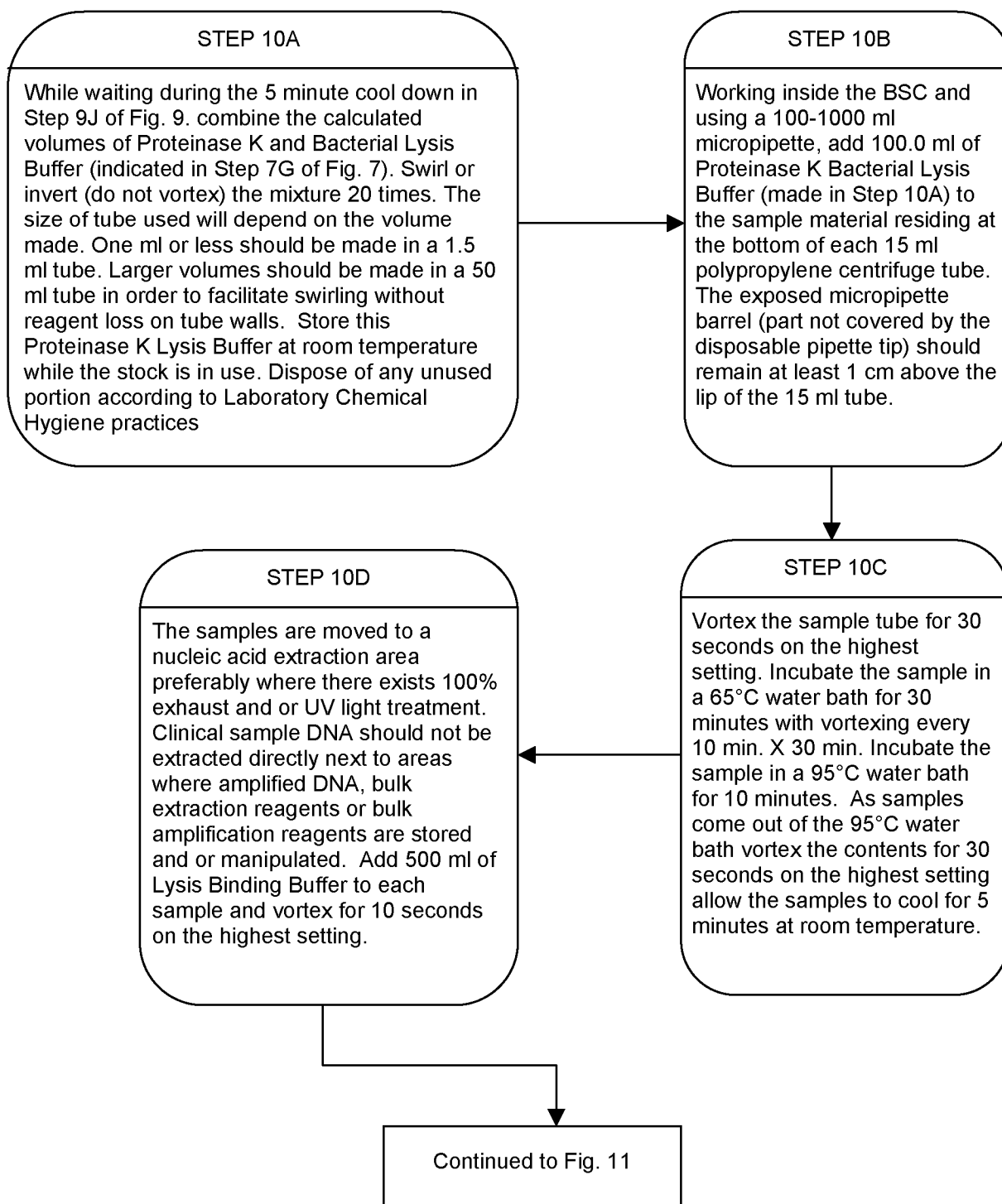
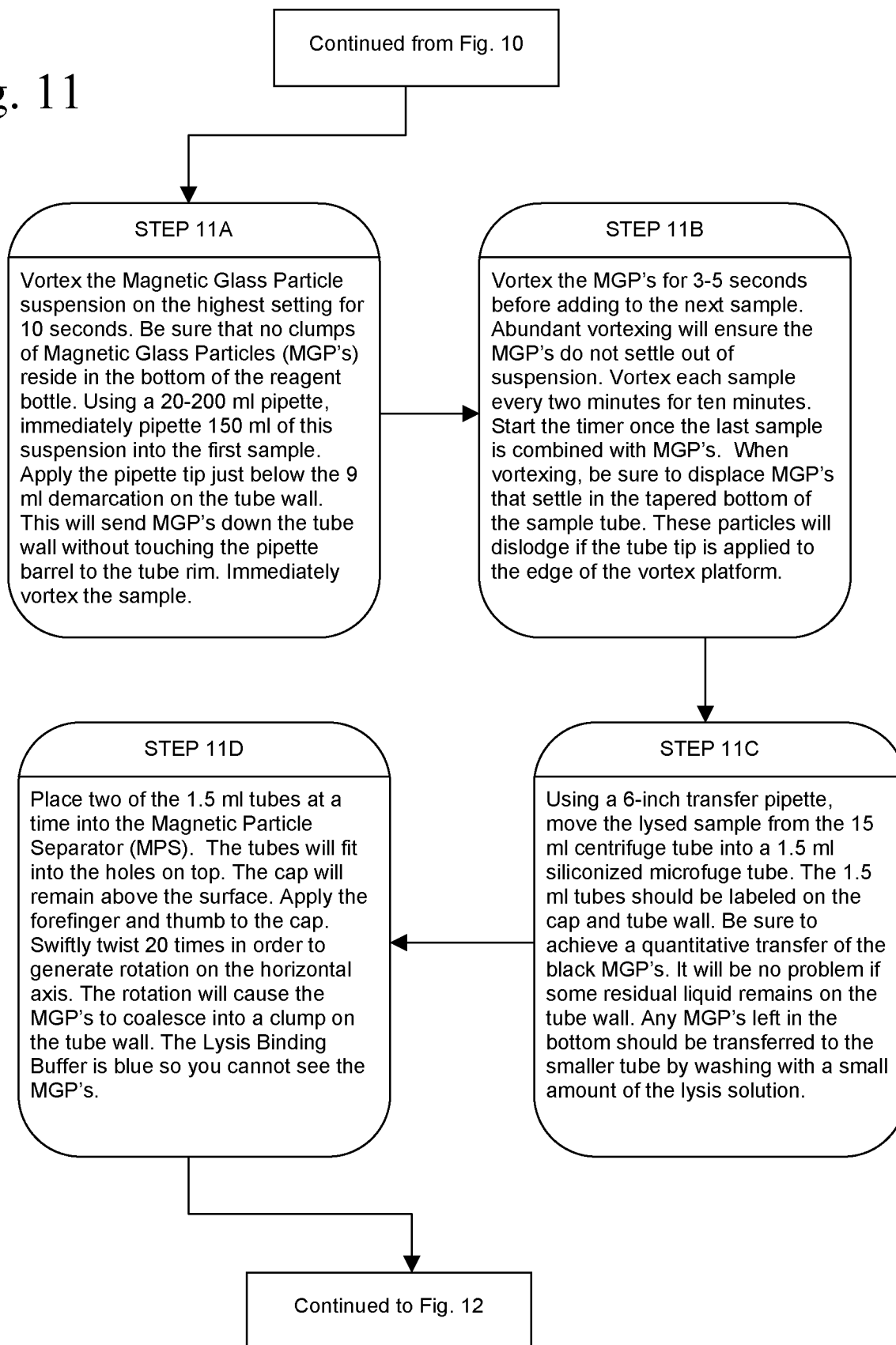


Fig. 11



Continued from Fig. 11

Fig. 12

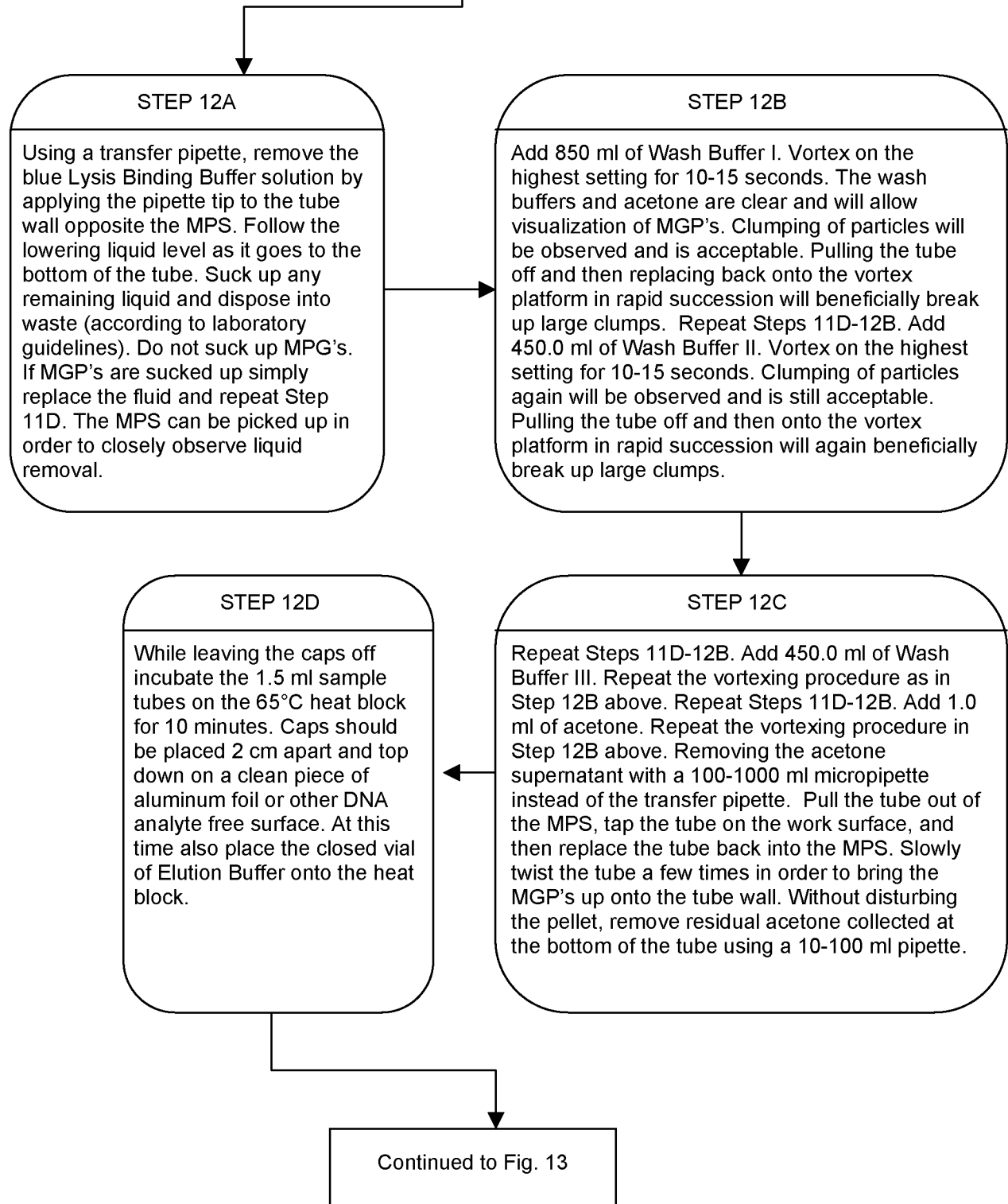


Fig. 13

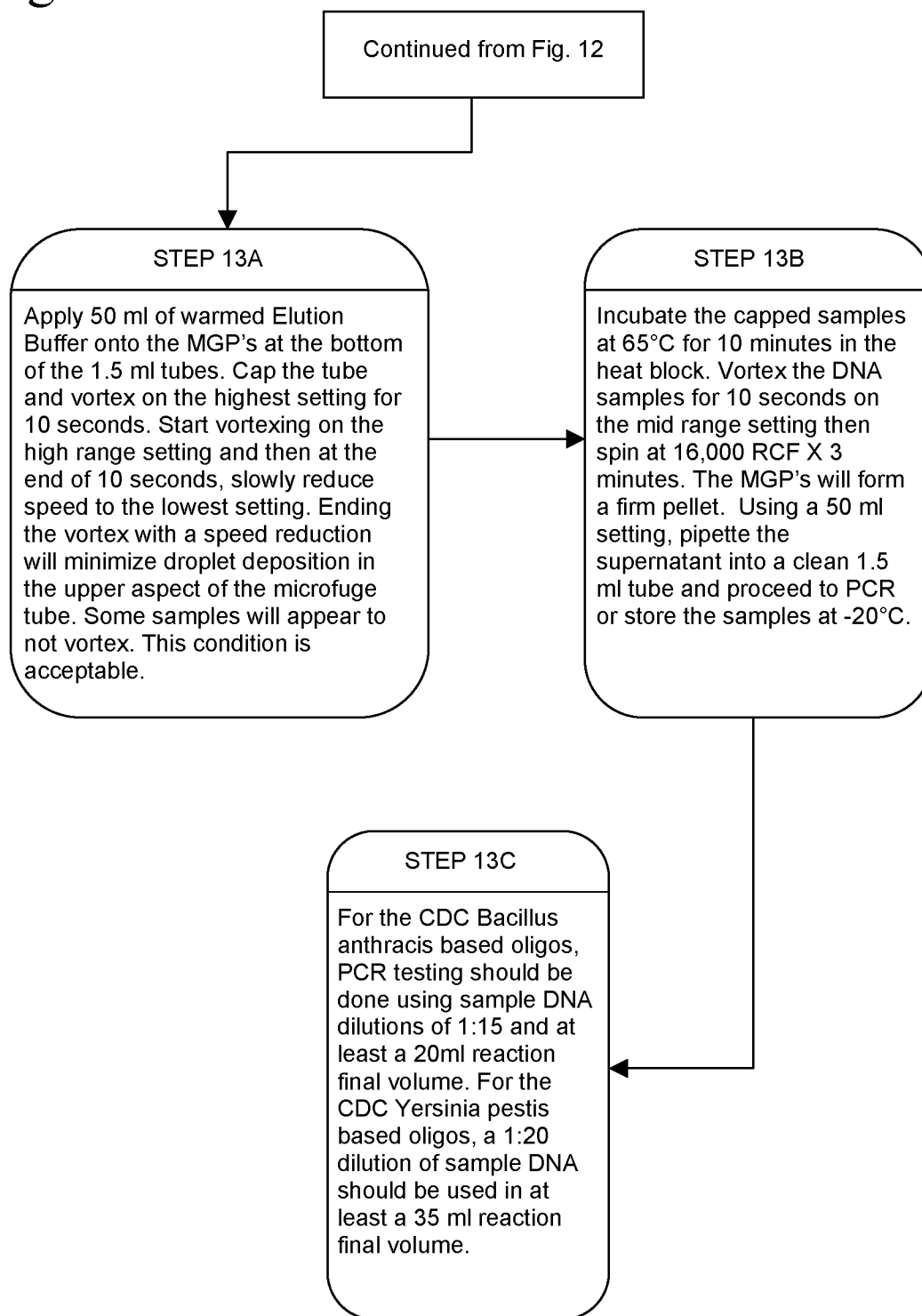


Fig. 14a

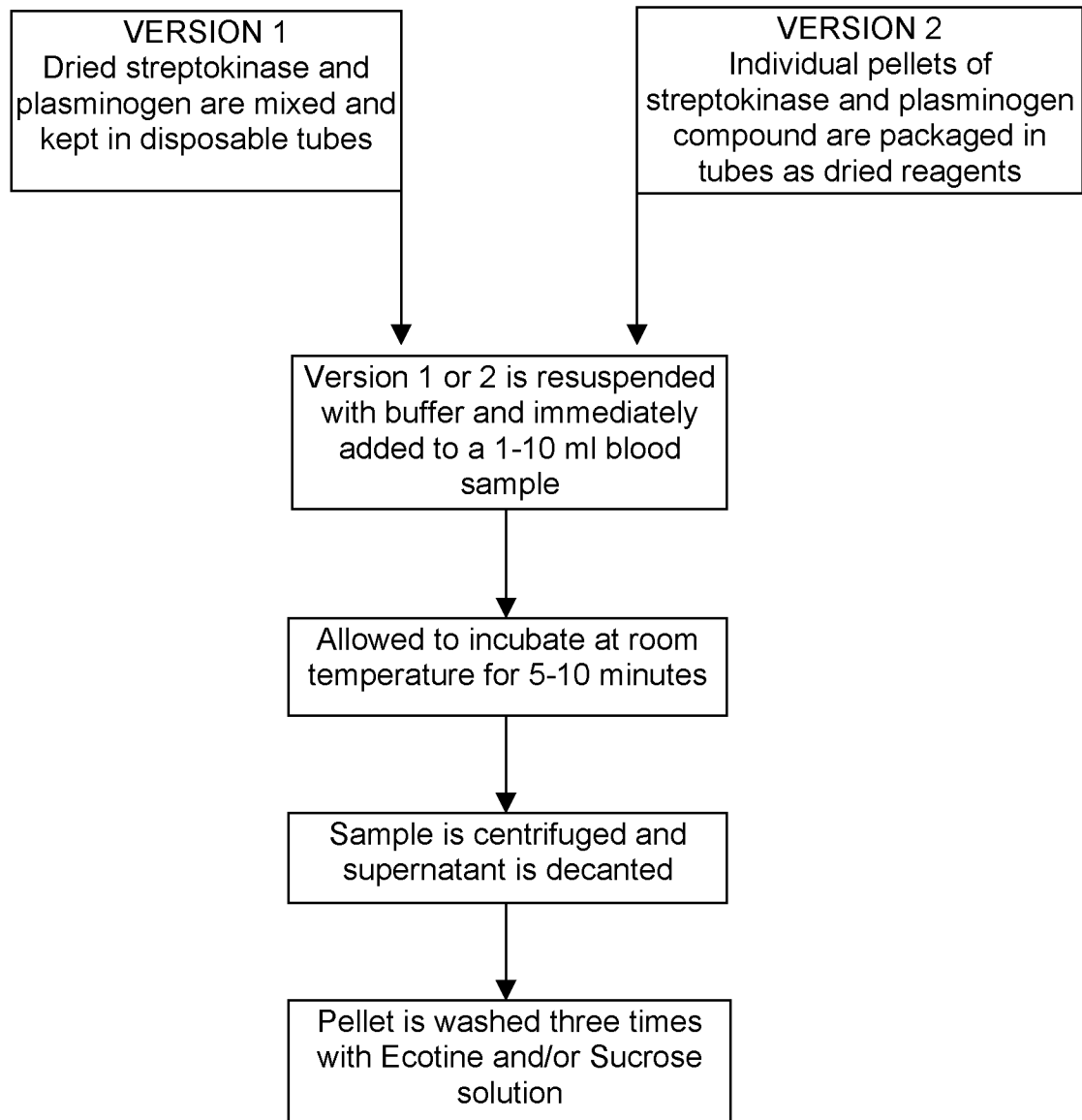


Fig. 14b

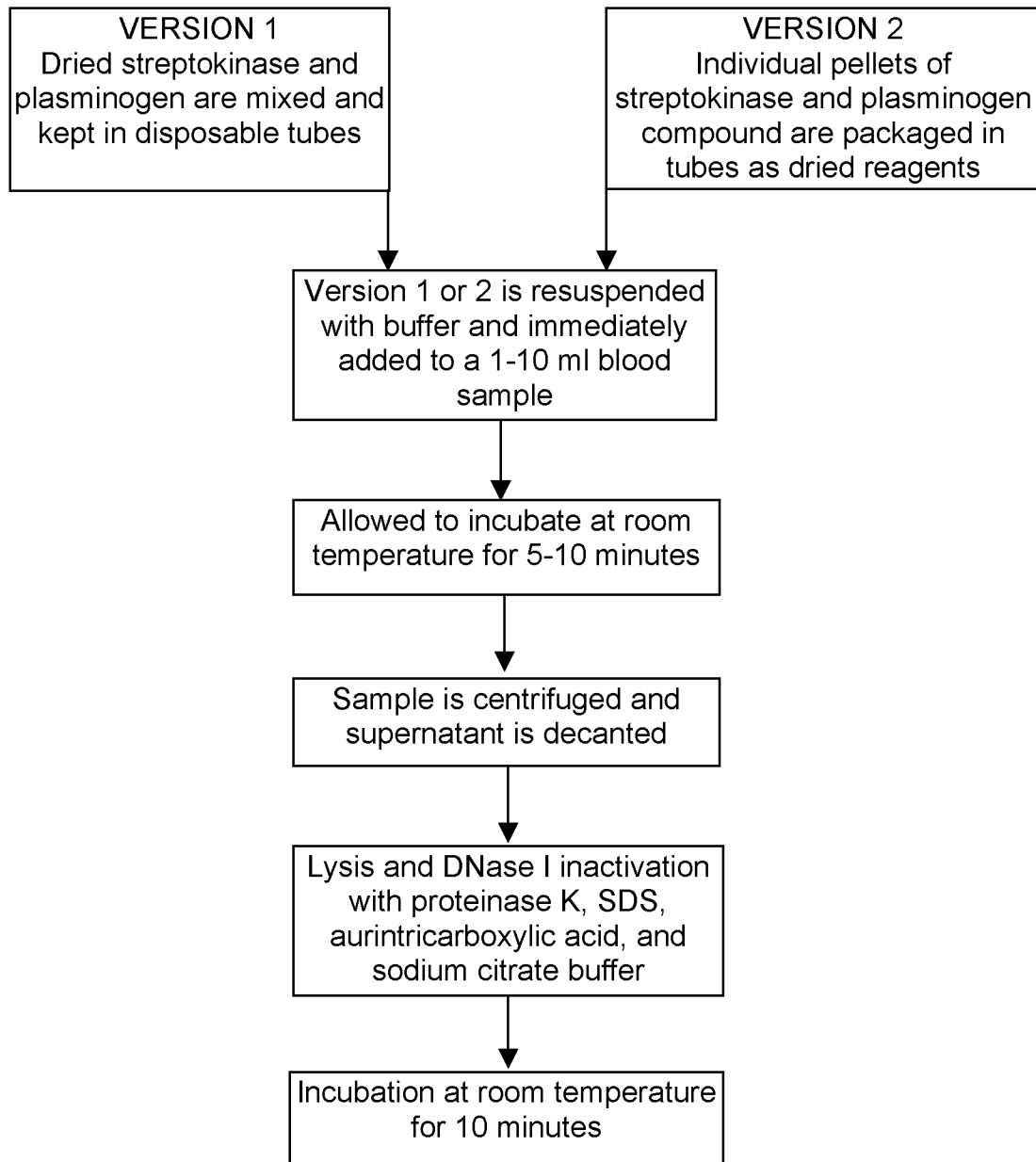


Fig. 15

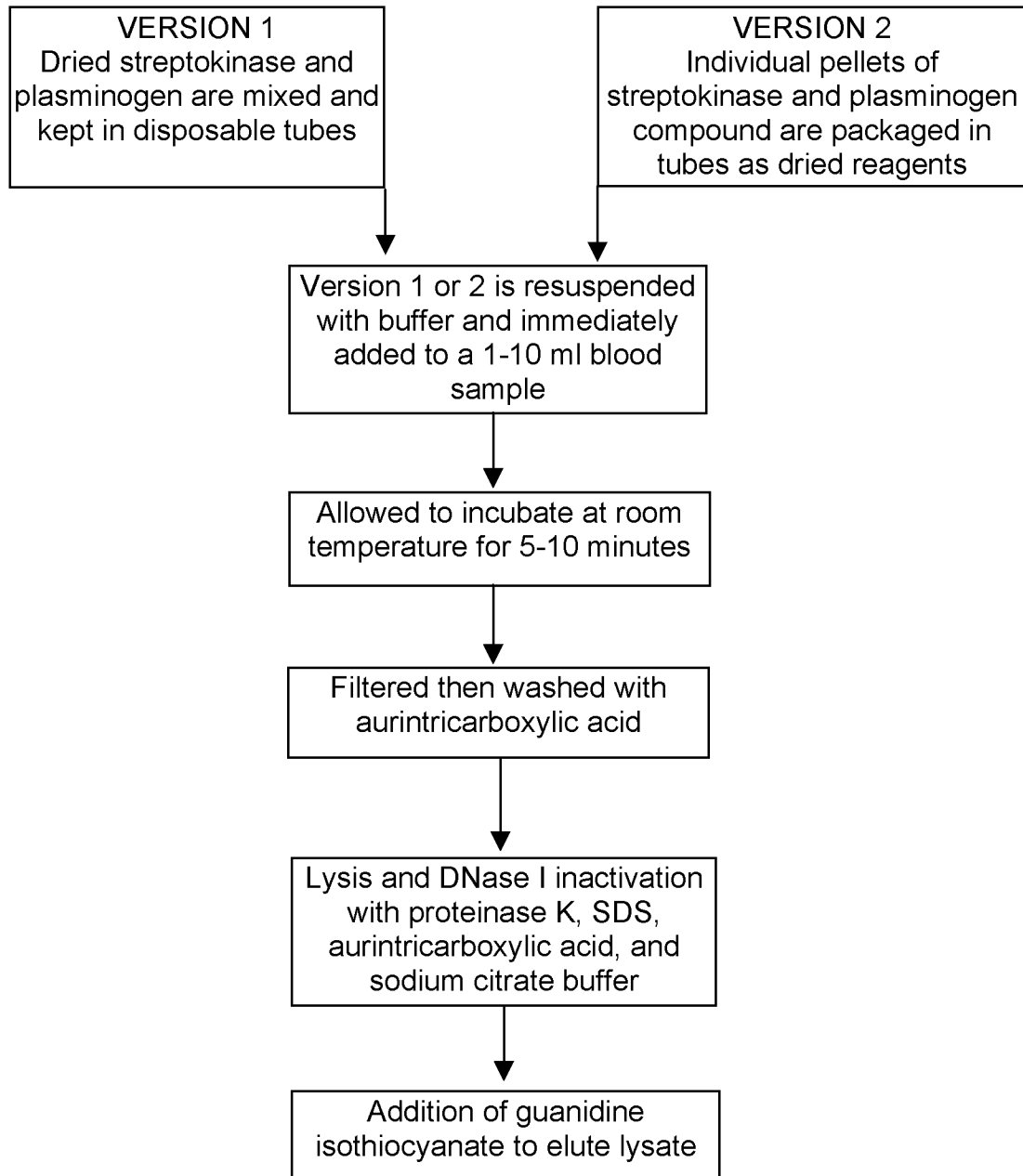


Fig. 16a

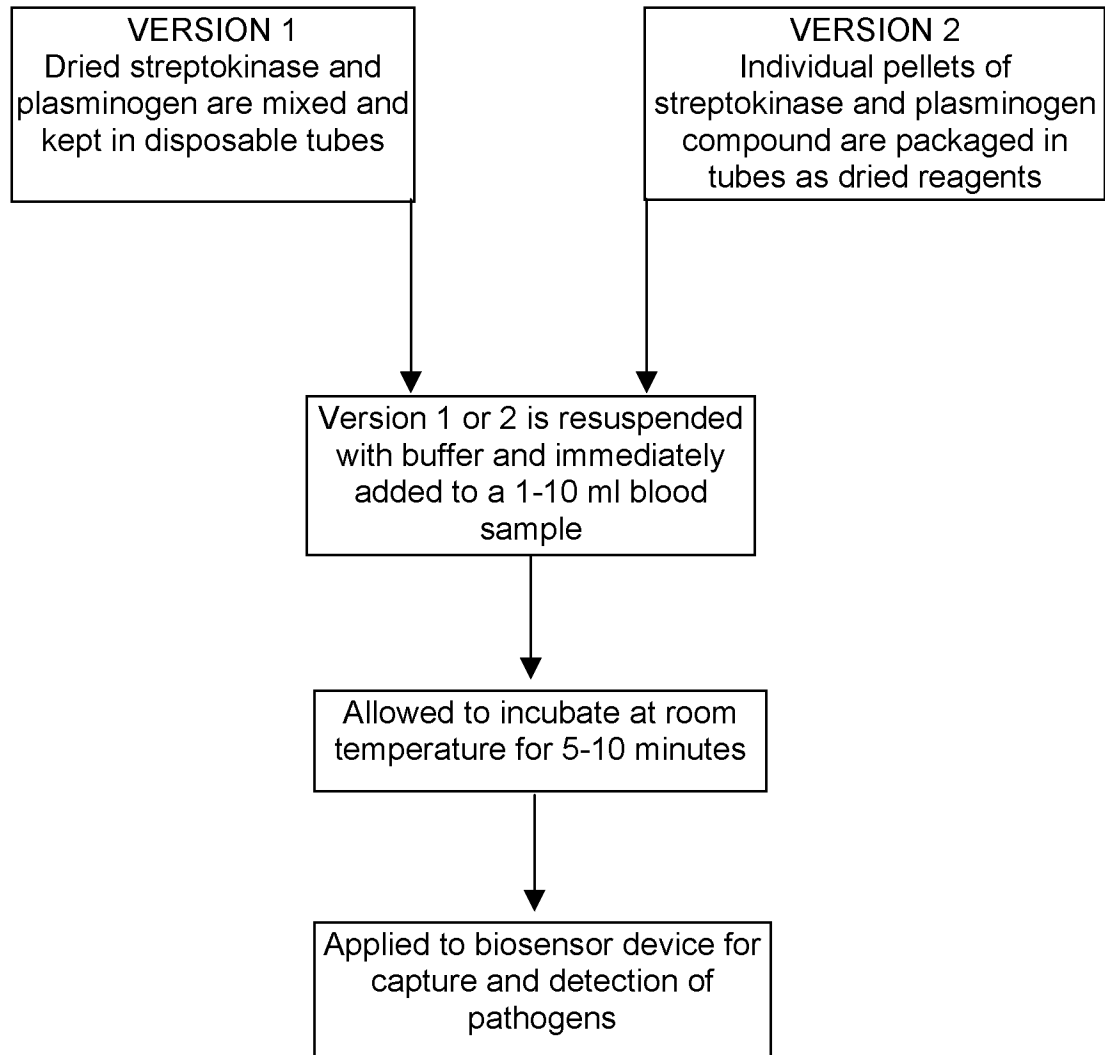




Fig. 16b

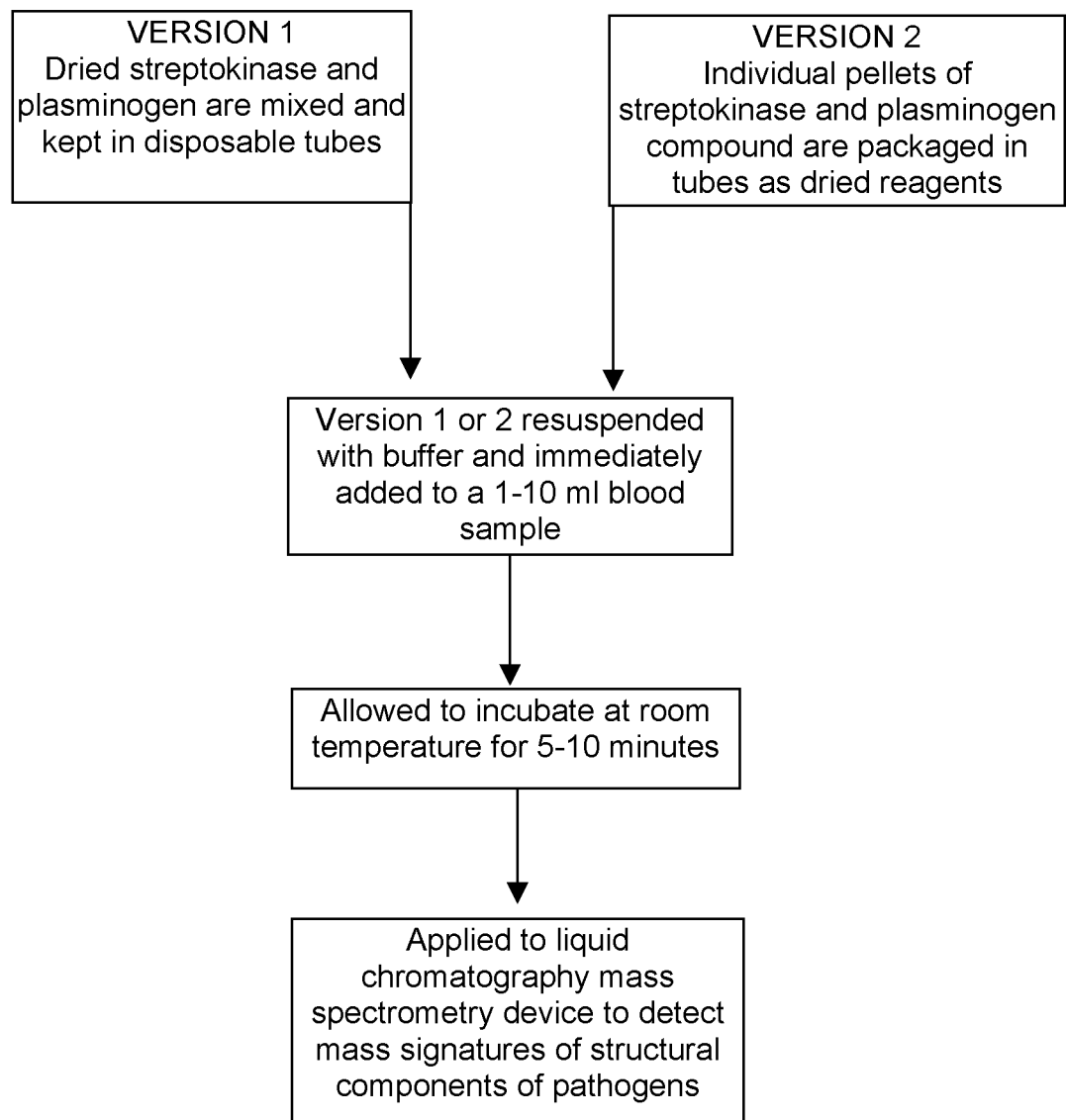


Fig. 17

**Noise band crossing points for blood samples spiked with *B. anthracis* and processed with plasminogen, streptokinase, phospholipase A<sub>2</sub>, DNase I, and lipase with centrifugation or filtration**

	<u><b>Centrifugation</b></u>			<u><b>Filtration</b></u>		
Amount <i>B. anthracis</i> Seeded (cfu)	Noise Band Crossing Points	Mean	Std. Dev.	Noise Band Crossing Points	Mean	Std. Dev.
≤ 0.01						
≤ 0.01						
≤ 1.0				40.33 39.89	40.11	
≤ 1.0				37.79	37.79	
≤ 2.0				40.36 37.69	39.03	
≤ 2.0	41.93 40.31	41.12				
≤ 5.0	40.47	40.47		37.90 37.70 37.79	37.80	0.10
≤ 5.0	38.11 40.36	39.24		36.45 36.09 36.81	36.45	0.36
≤ 50.0	37.53 36.24 37.90	37.22	0.87	35.75 34.12 34.98	34.95	0.82
≤ 50.0	36.45 38.15 38.49	37.70	1.09	35.24 34.18 34.68	34.70	0.53

## Fig. 18

### Sedimentation and solubilization of tissue aggregates from 6 ml blood samples exposed to various detergent and enzyme treatments

Enzyme treatments in a PBS/Triton X-100 buffer							
	Triton X-100 in PBS	Pl. <sup>c</sup> 1U	Ph. <sup>b</sup>	Pl. <sup>c</sup> 1U Ph. <sup>b</sup>	Dn. <sup>a</sup> 1mg	Dn. <sup>a</sup> 1 mg Ph. <sup>b</sup>	Dn. <sup>a</sup> 1 mg Pl. <sup>c</sup> 1U Ph. <sup>b</sup>
% Observable pelleted tissue aggregate post centrifugation	100	100	100	100	90	10	10
Time (min) to solubilization of visible tissue aggregate in BLB <sup>d</sup>	> 360	> 60	> 60	> 60	< 10	< 0.5	< 0.5

<sup>a</sup> DNase I from the Roche MagNa Pure LC DNA Kit III

<sup>b</sup> Phospholipase A<sub>2</sub>

<sup>c</sup> Plasminogen and 10K U streptokinase

<sup>d</sup> Bacterial Lysis Buffer from the Roche MagNa Pure LC DNA Kit III

Fig. 19

**Filtration characteristics of 6 ml blood samples exposed to various detergent and enzyme treatments**

Enzyme treatments in a PBS/Triton X-100 buffer							
	Triton X-100 in PBS	Dn. <sup>a</sup> 1mg	Dn. <sup>a</sup> 1 mg Ph. <sup>b</sup>	Pl. <sup>c</sup> 5U	Pl. <sup>c</sup> 5U Dn. <sup>a</sup> 1mg Ph. <sup>b</sup>	Pl. <sup>c</sup> 5U Dn. <sup>a</sup> 0.2mg Ph. <sup>b</sup>	Pl. <sup>c</sup> 10U Dn. <sup>a</sup> 0.2mg Ph. <sup>b</sup>
Not filterable	+	+	+				
Filterable with observable tissue aggregates				+		+	
Filterable with out observable aggregates					+		+

<sup>a</sup> DNase I from the Roche MagNa Pure LC DNA Kit III

<sup>b</sup> Phospholipase A<sub>2</sub>

<sup>c</sup> Plasminogen converted to plasmin with 10K U streptokinase